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Epigenetic drug therapy in the treatment of colorectal cancer

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Abstract

Colorectal cancer (CRC) is one of the most common cancers with a high rate of morbidity and mortality worldwide. The incidence of CRC is similar in men and women but is distributed uniformly globally. It has been demonstrated that epigenetic alterations which may cause changes in the expression of microRNA, DNA methylation and histone acetylation that results in inheritable modifications in gene expression in colorectal epithelial cells, plays a crucial role in the development of CRC. Recently, targeting epigenetic modification has emerged as a potentially important treatment approach in CRC. The US Food and Drug Association has approved the use of some epigenetic drugs that may be able to inhibit or reverse these alterations and also enhance sensitivity to chemotherapeutic agents and radiotherapy in CRC. In this review we have summarized the recent pre-clinical and clinical trial studies investigating the therapeutic value of using epigenetic drugs as novel therapeutic approach in CRC treatment.

Keywords: epigenetic, colorectal cancer, drug, DNA methylation, histone acetylation

Introduction

Colorectal cancer (CRC) is one of the most common cancers with a high rate of morbidity and mortality globally [1]. More than one million cases of CRC are diagnosed annually mainly in developed countries [2, 3]. Both men and women have a similar incidence rate for CRC[4]. The five-year survival rate is dependent on the stage at diagnosis, being approximately 90% in early stages of disease, and less than 10% in distant metastatic stage [2]. The incidence rate of CRC varies globally [5]. Some European countries, Australia, United States, Canada, New Zealand and Oceania are high rate regions. In contrast, in some countries of Africa, East and central Asia and parts of South America this rate is low [3, 5]. In addition to age, several risk factors are considered to be important for CRC: these include obesity, smoking, diabetes, high consumption of red meat and alcohol, inflammatory bowel disease, positive family history and some specific genetic factors[6]. There has been an increased use of colonoscopy, early detection and treatment, the CRC-related mortality has started to fall in high-income countries but the incidence rate has continued to increase in low-income countries [3]. It has been demonstrated that genetic and epigenetic alteration in colorectal mucosa cells can lead to neoplastic change [2].

Epigenetic change is an intracellular process that results in modifications of gene expression with no association of changes in DNA sequences [7]. Epigenetic alteration that consists of changes in the expression of microRNA (miRNA or miR), DNA methylation and histone acetylation play a crucial role in carcinogenesis from its initiation to subsequent metastasis [8]. In contrast to genetic changes, several epigenetic alterations can be reversed by treatment with particular category of drugs

that are termed epigenetic drugs and restore genes to their original expression and function [9]. Although there are several epigenetic drugs, few of them are approved by US Food and Drug Association (FDA). Overall, epigenetic drugs are categorized into two separate group: 1) Drugs that inhibit DNA methylation, and 2) Drugs that inhibit histone deacetylation. In the past decade, there has been some progress in understanding of how epigenetic alterations affect tumors pathogenesis. In addition to inhibiting cancer development, there is some evidence that treatment with epigenetic drugs can enhance chemo- and radio-sensitivity [8]. Recently, targeting epigenetic modification has been emerged as a potential approach to treatment in CRC and may represent a new ideas of personalized medicine. The purpose of this current study was to summarize the main results of studies used various epigenetic drugs for controlling cancer progression and treatment of CRC.

Molecular pathogenesis of colorectal cancer

Our understanding of the molecular aspect of CRC is evolving as new molecular detecting techniques have emerged. Epigenetic changes are becoming an important part of the pathogenesis CRC. Tens of thousands of mutations are reported in colon cancer cells and only a few of these are considered to be important driver or passenger mutations [10]. These mutations lead to genomic or epigenomic instability. Chromosomal instability (CIN), microsatellite instability (MSI) and aberrant methylation pattern are the 3 main genomic and epigenomic instabilities. These categories overlap with each other and according to recent advances in molecular analysis of CRC, it is not possible to distinguish these categories as independent and unique cause for CRC

progression. During CIN, the most common form of genetic instability in CRC, numerical chromosomal changes and structural aberrations are common findings [11]. Impairment in double stranded DNA repair mechanisms as well as deregulation of mitotic spindle, centromere and telomere dysfunction are major causes of CIN. Microsatellite instability (MSI) which is another common finding in CRC, is seen in approximately 15% of tumors. These tumors will mostly represent near normal karyotype and in some cases is accompanied by CIN [12]. It has been considered that dysfunction of DNA mismatch repair system is the main cause of MSI in CRC. Aberrant methylation or mutations of mismatch repair genes such as MLH1 is seen in MSI cancers [13]. CpG island methylator phenotype (CIMP) is the third cause of CRC pathogenesis, which demonstrates a global hypomethylation and also hypermethylation of CpG islands [14]. The defect in CIMP positive colorectal tumors may result in hypermethylation of the MMR genes promoters and therefore silencing of these repair genes [15].

Important enzymes involved in the epigenetic alterations of CRC

Some mechanisms are considered to be the most important epigenetic changes in CRC. CpG-island methylation is an important epigenetic alteration. The human genome has many CpG dinucleotide sites which are called CpG islands when become repeated in a specific sequence of DNA. CpG island are referred to those part of genome containing G or C content greater than 50%, with approximate equal number of CpG and GpC [16]. CpG island methylation refers to the methylation of cytosine nucleotides. This methylation process is catalysed by DNA methyltransferase (DNMT). There are 3 types of DNMT enzymes. DNMT1 is responsible for maintenance of DNA

methylation pattern during cell proliferation. This enzyme copies the CpG methylation pattern from parental DNA to daughter's DNA during S phase [17]. DNMT1 is also responsible for imprinting, tumorigenesis and embryogenesis [18]. It has also been shown that DNMT1 function is essential for progenitor cell function. Depletion of this enzyme will result in premature cell differentiation and tissue loss [19]. Recently the function of DNMT enzyme has emerged as being important in the determination of cancer prognosis and individualized treatment. As an example, cervical cancer cells express higher levels of DNMT1 in contrast to normal tissue and this is strongly associated with poor survival [20]. Another example for DNMT1 response to therapy is in CRC. It has been shown that DNMT1 disruption is associated with resistance to DNMT inhibitors [21]. Deregulation of DNMT1 activity is considered as an oncogenic phenomenon. Mutations in enzyme domains as well as activation or inactivation of alternative mechanisms targeting DNMT1 is responsible for epigenetic changes in cancer cells. Hyper activated signaling pathways such as PI3/PKB and Ras-AP1 as well as loss of inhibition of Rb and P53 pathways on DNMT1 are important mechanisms for promoting cancers [22]. DNMT3 is another family of DNMT enzymes, consisting of DNMT3a and DNMT3b and an inactive regulator, DNMT3L [23]. DNMT3a and DNMT3b cause DNA methylation, de novo, during germ cell differentiation [24]. Recently, it has been shown that the maintenance of methylation is not only provided by DNMT1; DNMT3 is an essential factor [23]. Regardless of methylation capacities, DNMT1 and both DNMT3a and DNMT3b can repress transcription partially through activation of histone deacetylase (HDAC). DNMT1 forms a stable complex with transcription factors such as HDAC1 and DNMT3 and DNMT3b will interact with HDAC through their ATRX-like PHD

domain [25]. HDACs are group of enzymes that remove the acetyl group from histones; allowing the histones wrap DNA more tightly. Tighter wrapping reduces the accessibility of DNA for transcription factors [26]. There are many proteins working together with HDACs in order to form a repressive complex. Methyl groups at CpG islands are the initiators of recruitment of HDAC complex [26]. Another mechanism is that HDAC, regulates the acetylation of important proteins such as P53, Sp3 and E2F. Deacetylation of DNA sequence specific transcription factors are also linked to reduced DNA transcriptional activity or binding [27]. The role of HDACs in cancer is still under active research and many mechanisms for cancer development are considered to involve HDACs. Aberrant recruitment of HDACs is often found in malignancies such as acute promyelocytic leukemia. HDACs expression is variable in different cancers. Transcriptional repression of tumor suppressor genes is another common mechanism in tumor development and progression [28]. Increased expression of HDAC1 is seen in colon cancer, breast and gastric cancer while overexpression of HDAC2 is mostly seen in cervical and colorectal carcinoma with loss of APC expression [27, 29-31]. Another group of enzymes that are important in epigenetic research are the Histone acetyltransferases (HATs). These enzymes acetylate histone proteins by transferring acetyl group from acetyl-CoA. Generally, histone acetylation is linked to transcriptional activation and euchromatin. Histone acetylation neutralizes histone charge, weakens histones and therefore reduce chromatin compaction. The acetylated histone can also work as a “histone code” which allows recognition sites for factors which are essential for gene expression or repression [32]. Aberrant HATs activities may result in several cancers, including colon and lung cancers [33]. As for HDACs, genes that encode HATs

can undergo mutation, translocation and amplifications. Missense mutations and truncating mutations in some HATs (p300) have been identified in CRC [34]. The other enzyme which is important in epigenetic modifications is histone demethylase. Histone lysine demethylase is a chromatin modifier which acts along with DNA methylation. Lysine specific demethylase 1 is a well-known example of histone demethylase which plays an important role during epidermal mesenchymal transition [35]. Absence of this enzyme may result in repression failure of E-cadherin transcription. Overexpression of this enzyme is linked to proliferation, invasion and metastasis in different cancers. It has been proposed that lysine specific demethylase 1 may stimulate colon cancer metastasis [36]. Another histone modifying enzyme is histone methyltransferase (HMT) which catalyze transfer of methyl group to histone protein residues. Inactivation of HMT genes such as SETD2 are considered as common event human cancers such as renal cell carcinoma [37]. HMT subunits such as SUZ12 and PCL3 over expression is reported in colon cancer [38, 39].

Epigenetic drugs in colorectal cancer

Advances in our understanding of the particular epigenetic alteration in conditions such as CRC have resulted in a new class of chemical compounds that have been termed epigenetic drugs. Some of these drugs have been used in CRC patients and cancer cell lines, as follows:

Histone deacetylase inhibitor (HDACi)

HDACi are a group of drugs that can be purified from natural sources or synthesized. Four major groups of this epigenetic drug category are benzamides, hydroxamates, cyclic peptides and aliphatic acids. It has been proposed that normal cells are resistant and tumor cells are sensitive to HDACi and undergo growth arrest or cell death. Upregulation of p21 and blocking cyclin/CDK complexes are mostly the cause of inhibited cell cycle arrest and differentiation by HDACi [40]. This drug category includes: suberoylanilide hydroxamic (vorinostat), panobinostat, resveratrol, carbamazepine, arginine butyrate, sodium butyrate, plitidepsin, valproic acid, entinostat, romidepsin, droxinostat, resminostat, belinostat and SB939. Liu et al. evaluated the anticancer activity of Largazole in vivo and in vitro. They studied its effects in several colon cancer cell lines and reported that this HDACi drug is active against several colon cancer cells. They also demonstrated that largazole inhibits HDAC in mouse with xenograft tumor tissue and tumor cell apoptosis and growth inhibition was prominent [41]. Munster et al. evaluated the effect of supra-therapeutic doses of vorinostat, on QTc interval in patients with advanced cancers including CRC. Electrocardiographic holter monitoring before administration and till 24 h after that revealed that administration of 800 mg vorinostat is not correlated with prolongation of the QTc interval [42]. Wilson et al. conducted the study to evaluate the safety of chemotherapeutic treatment consisted of vorinostat and 5-fluorouracil (5-FU) in patients suffered from metastatic CRC who previously failed 5-FU-based chemotherapy. This study was terminated because of poorly tolerated drugs combination [43]. Maximum tolerated dose (MTD) of vorinostat was assessed in combination with FOLFOX regimen that includes fluorouracil (FU), folinic acid, also known as leucovorin and oxaliplatin in twenty-one patients with

refractory CRC. Finally, 300 mg orally twice daily x 1week every 2 weeks was determined as a MTD of vorinostat in combination with FOLFOX regimen [44]. Vansteenkiste et al. with investigation of patients with refractory or relapsed cancer including CRC found that daily oral vorinostat regimen for 14 days/3 weeks is tolerable only at 200 mg twice daily, and no clinical responses were observed [45]. Hamberg et al. evaluated the feasibility of co-administration of panobinostat with ketoconazole as a CYP3A4 inhibitor in patients with cancer including CRC and results showed that this co-administration is feasible [46]. Fakih et al. investigated metastatic CRC patients who had chemotherapeutic response failure. Regarding the synergistic effect of combination of vorinostat and 5-FU in preclinical models, they administered low dose (800 mg) and high dose (1400 mg) of vorinostat combined with 5-FU. Finally results showed no clinically relevant activity for this regime [47]. In a study performed by Patel et al. in a twenty patients with CRC, was observed that taking 0.5-1 g of resveratrol orally makes sufficient dose to provide anti-carcinogenic effects in gastrointestinal tract. In addition, these dose of resveratrol resulted in 5% reduction in cancer cells proliferation [48]. Delius and colleagues aimed to diminish neurotoxicity of oxaliplatin by means of administration of carbamazepine in patients with advanced CRC. Finally they observed no clinical effectiveness [49]. Douillard and colleagues who have used high dose of arginine butyrate (ArgB) regimen in contribution with Interleukin-2 (18 MIU/m²) in six patients with advanced metastatic cancer, reported liver insufficiency and toxicity. They also observed that the MTD for ArgB was 3 g/kg/day[50]. In another clinical trial investigated the MTD of combination therapy with plitidepsin and bevacizumab in 13 patients with advanced cancer including CRC revealed that this regimen was well

tolerated and no unexpected toxicities were observed. In this clinical trial stable disease was the best response. They have concluded that 3.8 mg/m² of plitidepsin and 10 mg/kg of bevacizumab is tolerated in advanced carcinomas [51]. A regimen consists combination therapy of valproic acid with dose of 5.3 mg/kg and 11 mg/kg bevacizumab was observed to be safe with stable disease more than 6 months in patients with advanced malignancies including CRC [52]. Ree et al. conducted a clinical trial on 16 patients with gastrointestinal cancer including CRC to evaluate the MTD of vorinostat in combination with palliative radiotherapy. Results showed that administration of 300 mg vorinostat daily three hours before radiation is safe and well tolerated. As well biological activity of vorinostat when administered with pelvic radiotherapy was also confirmed in this study [53]. Mahalingam evaluated patients with advanced cancers including CRC and found that MTD of hydroxychloroquine and vorinostat was 600 mg and 400 mg orally once per day, respectively. Combination of hydroxychloroquine with Vorinostat did not significantly affect the pharmacokinetics of Vorinostat. They also observed that most of the evaluated patients did not benefit from combination therapy with this regimen [54]. Fakih and colleagues aimed to determine the MTD of vorinostat in combination with 5-fluorouracil (5-FU) and leucovorin. Results showed that the MTD of vorinostat when orally used in this combination therapy is 1700 mg daily × 3 or 600 mg twice a day × 3 days every two weeks [55]. Intermittent combination therapy of vorinostat and bortezomib was assessed in a study conducted by Deming et al. and was observed that the intermittent dosing protocol did not resulted in better tolerance to vorinostat. As well, the MTD of vornostat was determined as 300 mg orally twice daily on days 1–4 and 8–11 and intravenously use of bortezomib 1.3 mg/m² on days 1, 4, 8, and 11 of a 21 day

cycle [56]. Ryan et al. conducted a clinical trial study on 31 patients (2 CRC patients) with advanced tumors. They have used oral Entinostat as a HDAC inhibitor. They have stated that Entinostat half-life was up to 80 hours. Dose limiting toxicities were anorexia, fatigue, nausea and vomiting which were happened in 10mg/m² in the q14- day regimen [57]. Whitehead et al. treated their locally advanced and metastatic CRC patients with 13mg/m² epsipeptide intravenous infusion as a histone deacetylase inhibitor. Their patients have failed either one or two chemotherapy regimen. Six patients had treatment toxicities as thrombocytopenia, electrocardiographic ST-T changes, fatigue, nausea and vomiting, weakness, anorexia, fever and weight loss. They have showed that epsipeptide at the mentioned dose is ineffective in CRC patients with prior chemotherapy [58]. Howells et al. had evaluated the effect of SRT501, micronized resveratrol as an HDACi on CRC and hepatic metastasis. Daily dose of 5 g for 2 weeks were well tolerated and apoptosis marker were significantly increased in hepatic malignant tissue after administration [59]. Razak et al. used SB939 as a competitive histone deacetylase inhibitor in patients with advanced solid tumor including CRC. They have shown that this drug is well tolerated in advanced tumors and the toxicities were included as fatigue, vomiting, diarrhea and anorexia. The recommended phase II dose was 60mg on 5 consecutive days each 14 days [60].

DNA methyltransferase inhibitor (DNMTi)

DNMTis are a group of drugs that can inhibit DNA methylation activity of DNA methyltransferase and cause a reduction in genomic DNA methylation in some cancers. This class of epigenetic drugs can induce demethylation of some genes which

are silenced epigenetically. Several silenced genes in DNA repair, cell cycle and apoptosis including P15, P16, tissue inhibitor of metalloproteinase 3 and cyclin-dependent kinase inhibitor 1C are reported to be reactivated after administration of DNMTi [61]. 5-Azacytidine, 5-Aza-2'-deoxycytidine, also called 5-aza-DC or decitabine, hydralazine, procainamide and zebularine are included in this drug category. Braiteh et al evaluated the effect of administration of subcutaneous 5-Azacytidine as a hypomethylating agent for 10 days with therapeutic dose of oral valproic acid as a histone deacetylase inhibitor. Fifty five patients (11 patients with CRC) were enrolled and neutropenic fever and thrombocytopenia which were dose limiting toxicities occurred at a dose of 94 mg/m² for 5-Azacytidine. The safe dose of 5-Azacytidine was reported to be up to 75 mg/m². Their patients showed significant decrease in global DNA methylation and induced histone acetylation [62]. Li et al. evaluated the effect of 5-Azacytidine on 63 cancer cell lines including CRC. They have documented enrichment in immunomodulatory pathways including interferon signaling, cytokines, chemokines, antigen presentation and processing. Up regulation of AZA Immune gene set (AIM) after treatment was evident. According to this study, broad immune stimulatory is a result of DNA demethylating agent [63]. Cho et al. evaluated the effect of HDAC inhibitor sodium butyrate and demethylating agent 5-aza-DC along with radiation in colon and breast cancer cell lines survival. Survival was lower in 5-aza-DC or SB than radiation alone in CRC cell lines. The lowest survival was observed when combining 5-aza-DC and SB in combination with radiation. So, combination of these agents considered as effective agents in radio sensitivity in both colon cancer and breast cell lines [8].

Histone demethylase inhibitor (HDMi)

There are several inhibitors reported for histone demethylase. Polyamine and substrate analogs have been developed to target catalytic domains of histone demethylase. Re-expression of aberrantly silenced genes in cancer cells are one of the main functions of HDMis. Apoptosis has been reported to be induced in some cancers such as Ewing sarcoma [64]. Clorgyline, tranylcypromine, pargyline, GSK-J4, JIB-04, bizine, KDM5-C70 and GSK2879552 are drugs that are in this class. Han et al. used clorgyline as a lysine specific demethylase 1 to treat colon cancer lines. They found that the therapeutic efficacy of combining this drug with 5-Aza-CdR (5-Aza-2'-deoxycytidine) has synergic effects on enriching H3K4me2 and H3K4me1 and therefore reactivating aberrantly silenced genes. Many of the reactivated genes were in interferon signaling pathways [65].

Histone methyltransferase inhibitor (HMTi) and histone acetyltransferases inhibitor (HATi)

Histone methylation is important mechanism in many cellular and biological processes including cell cycle, development and DNA repair. Methylation of different histones may result in the suppression or derepression of different genes. In addition to methylation, other histone modifications include acetylation of lysine tail on histone H3. The acetylation leads to an open chromatin formation while deacetylation provide closed chromatin and inhibited transcription[66]. HMTi class consists of several drugs such as GSK343, GSK126, 3-Deazaneplanocin A, CPI360, EI1, UNC0224 and UNC0638. Curcumin and C646 are two drugs that act as inhibitors of histone

acetyltransferases. Until now, there are no pre-clinical and clinical trial studies investigating drugs of these two categories in CRC.

Other new epigenetic drugs and strategies for targeting colon cancer

HDACis and DNMTis as well as HMTis are well known epigenetic drug classes in CRC therapy. In contrast to these well-known drugs, there are new drug recently developed for cancer therapy. Bromodomain and extra-terminal motif (BET) are one of these newly emerged epigenetic drugs. BET proteins can regular various cellular functions and also play an important role in oncogene expression. BET usually deal with super enhancers which are a cluster of acetylated histones. These acetylated histones control oncogene expression. BET protein will interfere with these acetylated lysines and therefore remodel chromatin structure. BET inhibitors will suppress the effect of these enhancers and interact with transcriptional activity [67]. BET inhibitors has been successfully used in suppression of MAPK signaling in colorectal samples [68]. Also, Hu et al. has demonstrated that BRD4 which is one of the BET proteins are highly expressed in colorectal cancer tissue samples and reported that CRC proliferation will be reduced by inhibiting BRD4 [69]. Interestingly, a recent study by Hogg et al. has proposed that BET inhibitors will be more efficient when used in combination. While BET inhibitors require an intact and efficient immune system, using BRD4 inhibitors in combination with immune modulatory agents will provide better results in cancer therapy [70]. Another newly emerged anti-cancer agents are antagomirs. Antagomirs are miRNA inhibitors which can bind to a specific miRNA and alter its function. There are plenty of miRNAs introduced to have oncogenic activities. miRNA-21 is one of the

first miRNA studied for its effects on downregulation of tumor suppressor genes in CRC. Considering such an important effect has made miRNA-21 as a potential target for treating CRC. Song et al. has conducted a study to inhibit this miRNA in CRC. They have designed an antagomir which was complementary to miRNA-21 sequence. The antagomir could usefully inhibit angiogenesis and proliferation [71]. Another method which has been used for targeting miRNAs is using small interfering RNA (siRNA). These siRNAs will bind to the desired miRNA and lead to its breakdown. Locked nucleic acid (LNA) method is another term which is newly used in targeting miRNAs. LNA is a modified RNA nucleotide. These modified RNAs have been proven to be safe, stable and non-toxic for using as a good antisense based gene silencing approach. Nedaeinia et al. has also used LNA anti miR-21 in CRC cell lines and showed the same results as Song and colleagues [72].

As mentioned earlier, epigenetic drugs can induce different effects in cancerous cells. HDACi and DNMTi are both well known for their potential effect on activating the silenced genes in CRC. Nowadays, many new treatment strategies have been provided for different types of cancers which are using different types of epigenetic drugs in combination. One of the earliest and successful example of combination therapy was provided by Juergens et al. in lung cancer. They have proved that DNA methylation inhibitors in combination with histone deacetylase drug. They have stated that low-dose azacitidine and entinostat in patients with solid tumors will be well tolerated and provide durable responses in such patients [73]. Combination therapy of epigenetic drugs in colon cancer is also becoming an area of interest although there is not sufficient data about the promising clinical responses yet. One of the latest clinical trials which has

recently published their results about combination therapy in CRC patients is Azad et al. study. They have treated their patients with a demethylating agent (5-azacitidine) and a HDACi (entinostat) and reported greater DNA demethylation in those who had PFS above median [74]. The combination therapy of epigenetic drugs and immunotherapies in colorectal cancer. The tight link of epigenetics and immunogenicity is becoming more evident according to recent advances in epigenetic researches. Li et al. study has provided valuable evidence about the effect of low dose 5-AC on CRC cell lines and provided new information about immune genes which upregulate significantly by epigenetic therapy. This drug has also important in upregulating antigen presenting pathways [63]. Combination therapy in other CRC models has also proven that adding entinostat or azacitidine to two key checkpoint inhibitors, anti-PD-1 and anti-CTLA-4 inhibitors has significantly decreased tumor growth and metastasis [75]. Despite of favorable results in preclinical studies and as same epigenetic drug combination studies, epigenetic and immunomodulatory drugs combinations are also seeking further clinical investigation.

Conclusion

Colorectal cancer is a heterogeneous disease with distinct molecular and clinical features, which reflects the wide range of prognostic outcomes and treatment responses. It is now believed that among thousands of epigenetic alterations, a small subgroup of these may be considered as a CRC driver event. DNA methylation profiles have been the most widely studied in CRC, which includes a subset of patients with distinct molecular and clinical features, providing us with new potential biomarkers for

diagnostic, prognostic and therapeutic purposes. This development leads to discovery of new drugs and has opened a new therapeutic window for colorectal cancer patients. Although there are many studies available for some epigenetic drug classes such as DNMTis and HDACis, however, other classes such as Histone methyltransferase inhibitor (HMTi) and inhibitors of protein binding to methylated or acetylated histones are not widely involved in clinical trials. While cancer therapy regimens are becoming friendlier with using epigenetic drugs, however, there is still a long road ahead of researchers for studying the exact mechanism and safety of these new drug regimens. As most of these drugs are using in combination with previously well-known cancer drugs, paying close attention to the tolerability and cost effectiveness of these regimens needs further researches, further studies are need to validate emerging agents in the treatment of CRC patients

Conflict of interest

The authors have no conflict of interest to disclose

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Author and year (reference)	Drug class	Drug name	Study type	Aim	Outcome	Reported side effects
Han et al., 2013 [65]	DNMTi and HDMi	5-Aza-DC and clorgyline	Pre-clinical	Evaluation of therapeutic value of combined 5-Aza-CD (5-Aza-CdR) and clorgyline	This combination has synergic effects on enriching H3K4me2 and H3K4me1	-
Liu et al., 2010[41]	HDACi	Largazole	Pre-clinical	Evaluation of anticancer activity of largazole	Largazole, is active against several colon cancer cells and makes tumor growth inhibition and apoptosis	-
Munster et al., 2009 [42]	HDACi	Vorinostat	Clinical trial	Evaluation the effect of supratherapeutic dose of vorinostat on ECG	Vorinostat is not correlated with prolongation of the QTc interval	Nausea, cough, fatigue, dyspnea
Wilson et al., 2010 [43]	HDACi	Vorinostat	Clinical trial	Evaluation the safety of combining vorinostat with 5-FU	Poorly tolerated this combination resulted in study termination	Fatigue, mucositis and thrombocytopenia
Fakih et al., 2009 [44]	HDACi	Vorinostat	Clinical trial	Evaluation MTD of vorinostat in combination with 5-FU oxaliplatin and leucovorin	The MTD of vorinostat in this regimen was 300 mg orally times a day x 1 week every 14 days	Thrombocytopenia, fatigue neutropenia and GI toxicities
Vansteenkiste et al., 2008 [45]	HDACi	Vorinostat	Clinical trial	Evaluation of tolerability and clinical response of vorinostat	No clinical responses with using were observed and it was tolerable at 200 mg twice daily	Anorexia, asthenia, nausea, vomiting, thrombocytopenia, and weight loss
Hamberg et al., 2011 [46]	HDACi	Panobinostat	Clinical trial	Evaluation the safety of administration of panobinostat and ketoconazole	Co-administration of panobinostat with CYP3A inhibitors is feasible	Diarrhea, anorexia, vomiting, nausea, hypophosphatemia, myalgia and fatigue
Fakih et al., 2011 [47]	HDACi	Vorinostat	Clinical trial	Evaluation the efficacy of low and high doses of vorinostat combined with 5-FU	No clinically relevant activity was seen in this combination therapy	Fatigue, nausea, diarrhea hand and foot syndrome
Patel et al., 2010 [48]	HDACi	Resveratrol	Clinical trial	Evaluation of sufficient dose of resveratrol for anti-carcinogenic effect in gastrointestinal tract	The sufficient dose was 0.5-1 g orally per day	Nothing was seen

Delius et al., 2007 [49]	HDACi	Carbamazepine	Clinical trial	Evaluation the efficacy of using carbamazepine to decrease oxaliplatin-associated neuropathy	Administration of carbamazepine was not reduce oxaliplatin-associated neuropathy	Nausea, dizziness, headache, mnemonic problems and optical hallucinations
Douillard et al., 2000 [50]	HDACi	Arginine butyrate	Clinical trial	Evaluation of the tolerability of high dose of arginine butyrate combined with interleukin-2	This combination demonstrated to be highly toxic with liver insufficiency and toxicity	Fatigue and liver toxicity
Aspeslagh et al., 2016 [51]	HDACi	Plitidepsin	Clinical trial	Evaluation of the toxicity and tolerability of plitidepsin in combination with bevacizumab	This combination was well-tolerated and no unexpected toxicities were observed	Fatigue, myalgia, and elevated ALT
Wheler et al., 2014 [52]	HDACi	Valproic acid	Clinical trial	Evaluation the safety of combination of valproic acid and bevacizumab	This combination was safe with stable disease more than 6 months	Altered mental status, proteinuria and hypertension
Ree et al., 2010 [53]	HDACi	Vorinostat	Clinical trial	Evaluation the MTD of vorinostat combined with palliative radiotherapy	The MTD of vorinostat in this protocol was 300 mg daily, three hours before radiation	Fatigue, GI toxicities, anorexia, diarrhea, rash hyponatremia, and hypokalemia
Mahalingam et al., 2014 [54]	HDACi	Vorinostat	Clinical trial	Evaluation of the MTD and safety of vorinostat combined with hydroxychloroquine	The MTD of vorinostat was 600 mg orally once per day No significant changes occurred in PK of vorinostat	Diarrhea, nausea, weight loss, fatigue, elevation in Cr, AST, ALT and ALP, anorexia, rash anemia, neutropenia, cough, dyspepsia, thrombocytopenia, dysgeusia, seizure, headache, hypertension, xerostomia, and constipation
Fakih et al., 2010 [55]	HDACi	Vorinostat	Clinical trial	Evaluation of the MTD of vorinostat combined with 5-FU and leucovorin	The MTD of vorinostat when orally used in this combination was 1700 mg daily × 3 or 600 mg twice a day × 3 days every 14 days	Fatigue, anorexia, nausea, vomiting, hand and foot syndrome and mucositis
Deming et al., 2014 [56]	HDACi	Vorinostat	Clinical trial	Evaluation the tolerability of vorinostat combined with bortezomib	Using this combination therapy did not resulted in better tolerance to vorinostat	Thrombocytopenia, anemia, fatigue, nausea, vomiting, anorexia, constipation, diarrhea
Cho et al., 2009 [8]	HDACi and DNMTi	5-Aza-DC and sodium butyrate	Pre-clinical	Evaluation the effect of 5-aza-DC and sodium butyrate on radiosensitivity	This combination improve radiosensitivity in colon cancer cell line	-

Braiteh et al., 2008 [62]	DNMTi	5-azacytidine and valproic acid	Clinical trial	Evaluation the MTD and safety of 5-azacytidine when combined with valproic acid	This combination is safe at doses up to 75 mg/m ² for 5-azacytidine	Neutropenic fever, anemia, somnolence, tremor, nausea, vomiting, electrolyte imbalance
Ryan et al., 2005 [57]	HDACi	Entinostat	Clinical trial	Evaluation of the MTD and pharmacokinetics of oral entinostat	The half-life and MTD of entinostat was up to 80 h and 10 mg/m ² , respectively	Anorexia, fatigue, nausea and vomiting
Li et al., 2014 [63]	DNMTi	5-azacytidine	Pre-clinical	Evaluation of the expression of AZA Immune gene set in cancer cell lines	Up-regulation of AZA Immune gene sets after epigenetic therapy was observed	-
Whitehead et al., 2009 [58]	HDACi	Romidepsin	Clinical trial	Evaluation of response probability to romidepsin infusion	Administration of 13 mg/m ² romidepsin infusion on days 1, 8, and 15 of a 28 day cycle wasn't successful in metastatic CRC	Thrombocytopenia, ST-T changes in ECG, fatigue, nausea, vomiting, weakness, anorexia, fever and weight loss
Razak et al., 2011 [60]	HDACi	SB939	Clinical trial	Evaluation of safety and dose limiting toxicity of SB939	The recommended dose was 60mg for 5 consecutive days each 2 weeks	Fatigue, vomiting, diarrhea and anorexia
Howells et al., 2011 [59]	HDACi	Resveratrol	Clinical trial	Evaluation of safety, PK and PD of resveratrol	Daily administration of 5 g micronized resveratrol for 2 weeks was well-tolerated. Micronized resveratrol had higher C _{max} than non-micronized resveratrol	Anal pruritus, diarrhea, chills, nausea, peripheral neuropathy, rash, skin irritation and flushing

DNMTi: DNA methyltransferase inhibitor, HDMi: Histone demethylase inhibitor, HDACi: Histone deacetylase inhibitor, 5-FU: 5-fluorouracil, ECG: electrocardiogram, MTD: maximum tolerated dose, PK: pharmacokinetics, PD: pharmacodynamics, C_{max}: maximal plasma concentration, GI: gastrointestinal, Cr: creatinine, AST: aspartate aminotransferases, ALT: alanine aminotransferases, ALP: alkaline phosphatase

Figure 1.Pathogenesis of colorectal cancer.

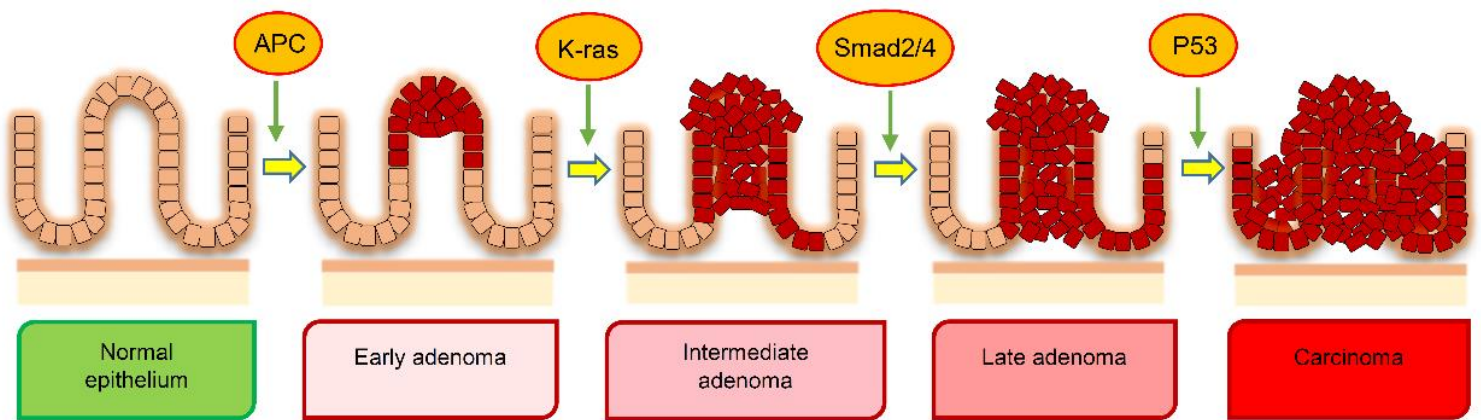


Figure 2.Epigenetic alteration in colorectal cancer. This figure illustrate the epigenetic alteration in human cells. The top figure shows histone modification. In this figure DNA methylation (Green dot) and histone tail acetylation (blue dot) has been shown. Histone acetylation has unfolded DNA to an open and transcriptionally active structure. The bottom part of this figure shows alteration in DNA methylation. The unmethylated CpG islands (Unfilled green dots) can be methylated by DNA methyltransferase (Green dots). Some microRNAs can inhibit this process and silence gene expression.

